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Leading Research Paper
Distraction Osteogenesis

A preliminary study of the effect of low intensity pulsed ultrasound on new bone formation during mandibular distraction osteogenesis in rabbits

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Abstract. This study assesses the effect of low intensity pulsed ultrasound (LIPUS) on new bone formation during mandibular distraction osteogenesis (DO) in rabbits. 24 rabbits underwent DO on the right side of the mandible. 12 rabbits received a daily 20-min LIPUS (1.5 MHz, 30 mW/cm^2) treatment on the first day of the distraction until they were killed at week 0 (immediately after the distraction), week 2 and week 4 after the distraction. Four rabbits were killed at each time point. The other 12 rabbits followed the same protocol without the ultrasound treatment. A plain radiography, a micro-CT scan, a microhardness test and a histological examination were used to evaluate new bone formation in the distraction gap. At week 0 and week 2 after the distraction, the treatment groups showed higher radiopacity and microhardness ($p < 0.05$), and more bone formation was detected by the histological examination. At week 4 after the distraction, there was no statistical difference between the two groups. In this study, LIPUS accelerated new bone formation during the distraction period and 2 weeks after the distraction, which implies that the effective time for using LIPUS is in the early stage of DO.

Keywords: Low intensity pulsed ultrasound; bone formation; distraction osteogenesis; mandible.

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Distraction osteogenesis (DO) has the unique ability to gain new bone and simultaneously expand the surrounding soft tissues without a donor site. It has achieved worldwide acceptance and great success in the treatment of numerous congenital and acquired craniofacial skeletal anomalies[4,15]. DO is a long term treatment, and it commonly takes 2–3 months for craniofacial DO[20]. The rate-limiting step is the long waiting period for new bone formation, which takes at least two-thirds of the whole treatment time. Great interest has been focused on reducing the treatment time by accelerating new bone formation during DO.

Low intensity pulsed ultrasound (LIPUS) is a form of mechanical energy that is transmitted through living tissues as acoustic pressure waves. Many studies have found LIPUS to be effective in the acceleration of fracture healing[16]. Two prospective, randomized, double-blind, placebo-controlled clinical trials have shown the same 38% reduction of healing time for tibial[9] and radial[11] fractures. The US Food and Drug Administration approved the use of LIPUS for the treatment of fresh fractures in 1994 and for the treatment of nonunion in 2000.

Several authors have reported the positive effects of LIPUS on long bone DO[2,17,19]. Few studies have evaluated the effect of LIPUS on membranous bone DO. EL-BIALY et al.[8] found that LIPUS accelerated new bone formation in a rabbit mandibular DO model, but SCHORTINGHUIS et al.[18] showed that LIPUS did not appear to stimulate bone formation in the severely resorbed vertical distracted human mandible. More investigations are required to clarify the effect of LIPUS on membranous bone DO. The aim of the present study is to assess the effect of LIPUS on new bone formation during mandibular DO in rabbits.

Materials and methods

24 skeletally mature, male, New Zealand, white rabbits weighing 3.5–4.0 kg underwent DO on the right side of the mandible. In brief, anaesthesia was induced by intramuscular injections of ketamine (35 mg/kg) and diazepam (5 mg/kg). The skin on the right side of the mandible was shaved and disinfected with iodine solution, and the submandibular incision was 3 cm long. Once the mandibular body was exposed, the complete osteotomy was made just anterior to the first premolar straight down to the border of the mandible using a fissure bur. The custom-made distractor, a modification from an orthodontic palatal expansion screw (Hyrax, Germany) (Fig. 1), was fixed to the mandible with 4 self-tapping titanium microscrews, and the distracting direction of the distractor was made parallel to the long axis of the mandibular body and perpendicular to the osteotomy line. The wounds were closed in layers. Postoperative care included wound care, and intramuscular injections of penicillin G sodium (0.5 million units) and acetaminophen (75 mg) each day for 3 days. After a 3-day latency period, the distraction was started at a rate of 0.5 mm/12 h for 10 days.

24 rabbits were randomly divided into two main groups: the ultrasound treatment group and the control (without ultrasound treatment) group ($n = 12$). 4 rabbits from each group were killed at weeks 0, 2 and 4 after the distraction (week 0 means immediately after the distraction without a consolidation period).

Ultrasound treatment

A commercial ultrasound device was used. The Sonic Accelerated Fracture Healing System (Exogen Inc., Piscataway, NJ, USA) provided LIPUS with a 1.5 MHz frequency, modulated at 1 KHz with a signal burst width of 200 ms and an intensity of 30 mW/cm^2. A single 20-min treatment to 12 rabbits per day started on the first day of the distraction and continued until they were killed at weeks 0, 2 and 4 after the distraction (Fig. 1).

Plain radiography

After the animal was euthanized with an intravenous injection of sodium pentobarbitone (1 ml/kg), its mandible was removed and separated at the synthesis, and a lateral film of the hemimandible (Kodak, Occlusal film, Ultra-speed, USA) was taken (10 mA, 50 KVP, 0.26 s, 12 in. FFD) with an aluminium step-wedge using an X-ray machine (Gendex, IL, USA) and processed by an automatic film processor (Dent X 9000, DentX/Logetronics GmbH, Germany). The films were transformed into digital images by a digital camera (JVC TK-C1380, Tokyo), and quantitative analysis was carried out with the software (Image Pro Plus 5.0, Media Cybernetics Inc., USA) to measure the mean grey level of the distraction gap that indirectly represents the projectional bone mineral density.

When the plain radiography was done, the specimen was cut from the distracted hemimandible including the distracted regeneration tissue and parts of the original bone, which was anterior and posterior to the distraction gap. Another horizontal cut along the long axis of the mandible body further bisected the specimen into upper and lower parts, and all the specimens were saline-soaked and frozen at −80 °C for preservation before additional testing.

Micro-CT

The lower part of the specimen was scanned transversely with a section of 200 μm thickness (μCT20, Scano Medical AG, Switzerland). The bone volume fraction (%, bone volume/total volume) was calculated with software (Revision 3.1, Scanco Medical AG, Switzerland).

Microhardness test

The upper part of the specimen underwent a microhardness using a Microhardness Tester (Buehler Micromed, England), and the Vickers microhardness (kg/

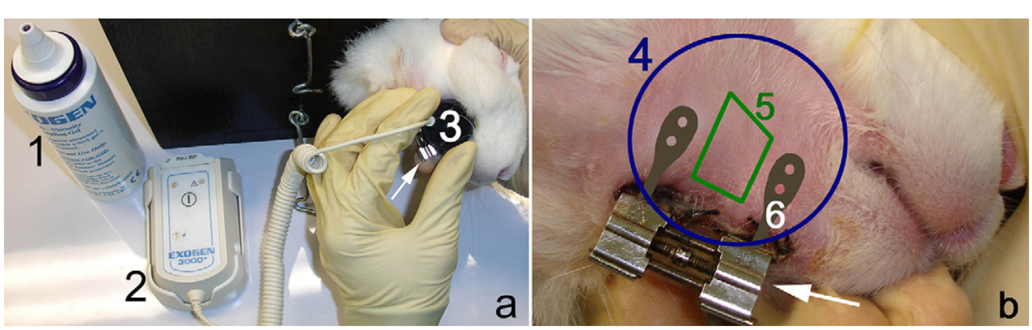

Fig. 1. Application of LIPUS. (a) When the LIPUS treatment was applied, ultrasound coupling gel was used. (1) Ultrasound coupling gel. (2) LIPUS device. (3) Ultrasound transducer head. (b) A simulation image shows the locations of (4) the ultrasound transducer head covering area, (5) distraction gap and (6) distractor leg. The arrow shows the custom made distractor.